Control points in early T-cell development

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Intrathymic T-cell differentiation involves the generation, expansion and selection of distinct T-lymphocyte subsets. While positive and negative selection have been a focal point of T-cell development, these events represent the final stages in a complicated sequence of differentiation steps. Here, Dale Godfrey and Albert Zlotnik summarize recent advances in our understanding of early T-cell development and describe five 'control points' that identify key events in this sequence.

Intrathymic T-cell development requires a continuous input of precursor cells from either the fetal liver or the bone marrow (reviewed in Refs 1, 2). The subsequent differentiation sequence eventually leads to the production of mature, self-tolerant, self-major histocompatibility complex (MHC)-restricted T lymphocytes. This overall process has been extensively reviewed elsewhere[3–5] and can be briefly summarized using a developmental pathway based upon CD4 and CD8 expression, which divides thymocytes into four main subsets. The $CD4^-CD8^-$ double negative (DN) cells, which are among the earliest subsets, are mostly surface CD3/T-cell receptor (TCR) negative, but are in the process of rearranging their TCR genes. These give rise to the $CD4^+CD8^+$, double positive (DP) subset, which express low to intermediate levels of CD3/TCR-αβ. At this stage, thymocytes are subject to positive and negative selection (also reviewed in Refs 6–9). Surviving cells down-regulate either CD4 or CD8 and mature into functional $CD4^+CD8^-$ or $CD4^-CD8^+$ T cells.

While the mechanisms of positive and negative selection have been the main focus of research into T-cell development over the last few years, events occurring prior to the DP stage are less well-defined. These include some key steps, including initial expansion, lineage commitment and regulation of TCR gene rearrangements. Collectively, these 'early' events consume much of a developing T cell's time in the thymus. The aim of this review is to define a series of 'control points' that regulate the progression of thymocytes from the earliest subset to the DP stage. Control points define steps which are dependent on the thymic microenvironment, as conceptualized previously[10]. This review is primarily focused on adult mouse thymocyte differentiation which is similar, although not identical, to embryonic mouse T-cell differentiation (reviewed in Ref. 5). Although human thymocyte development follows a similar sequence of differentiation events (reviewed in Ref. 11), a detailed comparison of early control points between the mouse and human models is difficult due to differences in the pattern of cell surface markers expressed during the early stages.

Significant progress in our understanding of the early events in thymocyte development has been made since the observation by Fowlkes *et al.*[12] that the $CD4^-CD8^-$ DN subset contained T-cell precursors capable of repopulating irradiated thymuses. It became evident that DN thymocytes were heterogeneous and could be subdivided based upon the expression of a diversity of markers including heat stable antigen (HSA), CD44 (Pgp-1), CD25 (interleukin 2 receptor α-chain (IL-2Rα)), Mel-14 and CD5 (Ly-1) (reviewed in Refs 10, 13). Of particular significance was the observation that the DN thymocyte population also encompasses some mature $CD3^+$ cells, including thymocytes expressing either the TCR-γδ or the TCR-αβ (reviewed in Ref. 2). The latter subset of TCR-$\alpha\beta^+$ DN thymocytes represent an alternative T-cell development pathway, rather than an intermediate stage in mainstream $\alpha\beta^+$ T-lineage development. It then became apparent that precursor thymocytes should be classified as $CD3^-CD4^-CD8^-$ triple negative (TN) cells. These can be further subdivided, and the two most commonly used markers are CD25[14,15] and CD44[16] (reviewed in Refs 3–5, 10). These, along with c-kit[17,18], the receptor for stem cell factor (SCF), provide the basis for our proposed four-subset model of early thymocyte development, which can be summarized as follows: $CD4^{lo}CD44^+CD25^-c\text{-}kit^+ \rightarrow CD44^+CD25^+c\text{-}kit^+TN \rightarrow CD44^-CD25^+c\text{-}kit^{lo}TN \rightarrow CD44^-CD25^-c\text{-}kit^{lo}$ $CD4^{lo}CD8^{lo}$ (Fig. 1). This model is based upon an original version described by Pearse *et al.*[19] and more recent reviews by Nikolic-Zugic[4] and Shortman[10]. The progression from one subset to the next appears to be strictly regulated by the thymic microenvironment, and each step involves at least one putative thymic control point.

Control point 1: commitment to the T-cell lineage

For some time, the earliest thymic precursors were thought to be $CD44^+CD25^-TN$. These cells were shown to have the greatest reconstitution potential[13,19,20] and were among the earliest to appear during embryogenesis or thymic repopulation following irradiation[21–23]. However, they were already partially rearranged at the TCR-β locus[19], which prompted the search for an earlier subset that had not yet initiated TCR-β gene rearrangement and were more similar to bone marrow-derived thymic precursor cells. Two years ago, Wu *et al.* reported an earlier subset, characterized by low levels of CD4 expression[24]. These were entirely unrearranged at the TCR-β and -γ loci, and upon intrathymic transfer mediated a slow but extensive reconstitution of both αβ and γδ lineage T cells.

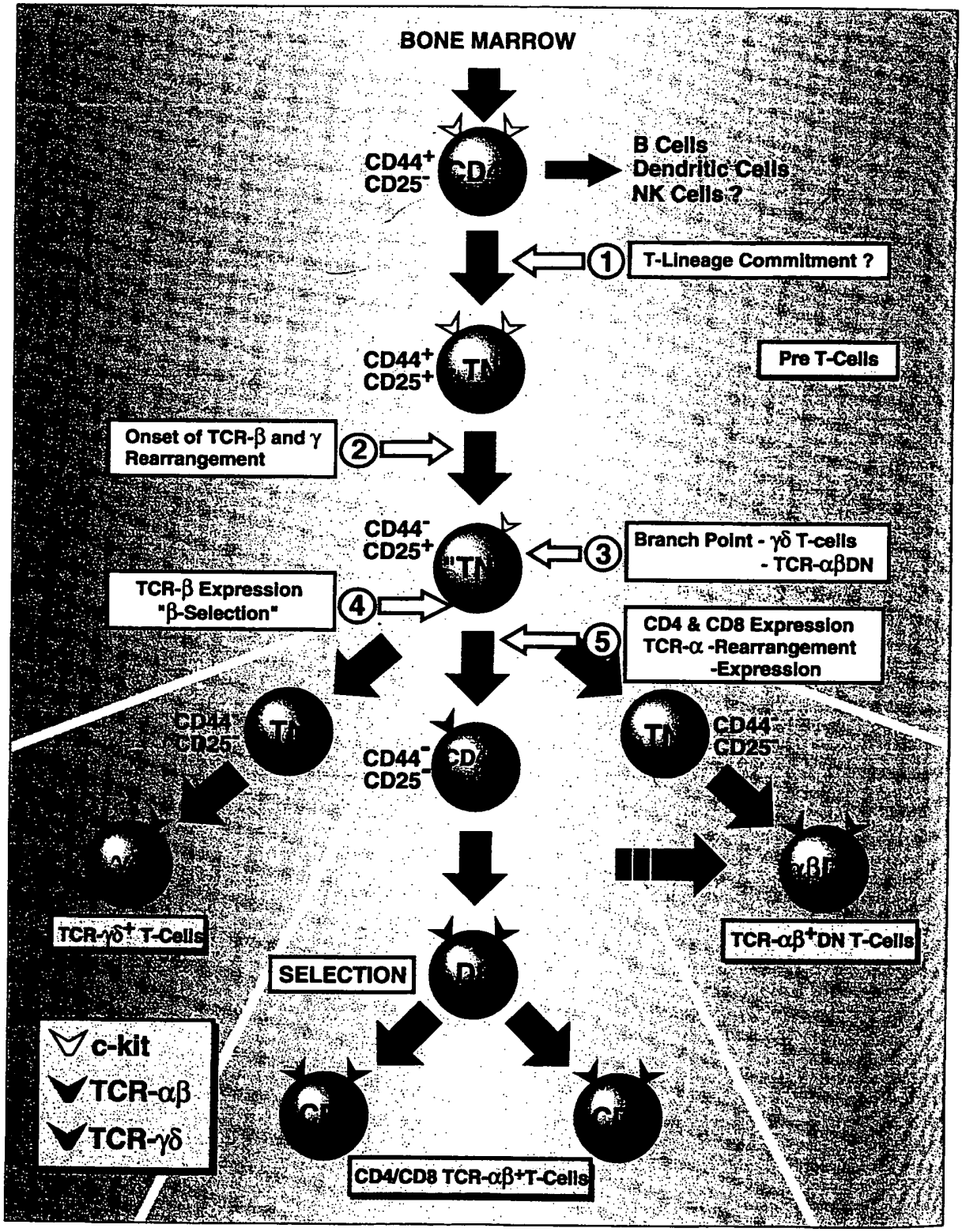

Fig. 1. *Control points in early T-cell development. This differentiation pathway describes T-cell development in the adult mouse thymus, focusing on events leading up to the CD4⁺CD8⁺ stage. Some subsets beyond the CD44⁻CD25⁻CD4^lo CD8^lo stage have been left out for the sake of simplicity. Two hypothetical branch points for the TCR-αβ⁺DN cells are shown: (i) these cells may branch off at the CD44⁻CD25⁺TN stage, which may subsequently involve TCR-α rearrangement and expression prior to maturation; (ii) cells of this phenotype may branch from the main pathway after TCR-αβ expression (dashed arrow). Abbreviations: DN, CD4⁻CD8⁻ double negative; DP, CD4⁺CD8⁺ double positive; TN: CD3⁻CD4⁻CD8⁻ triple negative; TCR, T-cell receptor; 'TN' refers to the possibility that these cells may express low levels of CD3/TCR-β.*

The discovery of the CD4^{lo} cells also provided evidence that, in contrast to hemopoietic stem cells of the bone marrow, the first cells in the thymus are no longer multipotential[25]. These cells were able to repopulate the T- and B-lymphoid components of irradiated mice, but possessed no myeloid or erythroid precursor activity, suggesting that they were lymphoid-committed stem cells[25]. More recently, this group reported that the CD4^{lo} precursors can also generate thymic dendritic cells (DC)[26]. The possibility remains that the CD4^{lo} precursors are heterogeneous and include distinct precursors for each lineage, since a clonal assay for the development of these cells has not yet been established. However, analysis with an extensive panel of monoclonal antibodies (mAb) has failed to reveal heterogeneity within this population[24,25].

The next step in the developmental sequence is therefore expected to be the CD44$^+$CD25$^-$TN. However, when these cells were screened for c-kit expression, which was previously defined as a marker of early thymocytes[17], only a subset were found to be c-kit$^+$ (Ref. 18). These c-kit$^+$CD44$^+$CD25$^-$ cells were germline at both their TCR-β and -γ loci, and upon re-examination, were found to express low levels of CD4, making them indistinguishable from the CD4^{lo} pre-TN cells described above (D.I. Godfrey, J. Kennedy, P. Mombaerts, S. Tonegawa and A. Zlotnik, unpublished). Conversely, the c-kit$^-$CD44$^+$CD25$^-$TN cells were rearranged at the TCR-β locus and spontaneously became TCR-αβ$^+$DN after overnight culture. Additionally, the c-kit$^-$CD44$^+$CD25$^-$TN cells also expressed CD38, a marker which we have found to be closely associated with the TCR-αβ$^+$DN lineage (A. Bean, D. Godfrey, L. Santos, M. Howard and A. Zlotnik, unpublished). These findings suggest that the real precursors within the CD44$^+$CD25$^-$ subset are the same as the CD4^{lo} pre-TN. Therefore, we have not included a CD44$^+$CD25$^-$TN stage in our model (Fig. 1) and propose that CD4^{lo} pre-TN give rise directly to CD25$^+$TN. There is presently no information on the mechanism(s) regulating this transition, nor on cytokine responsiveness or production by CD4^{lo} pre-TN cells. However, the expression of c-kit by these cells[17] suggests they might respond to SCF, perhaps in conjunction with IL-7, as has been described for the CD44$^+$CD25$^+$TN (Table 1). In accordance with this, the ability of these CD4^{lo}CD44$^+$CD25$^-$ cells (described as CD44$^+$CD25$^-$ 'TN') to repopulate fetal thymic lobes *in vitro* can be inhibited by a neutralizing anti-c-kit mAb (D.I. Godfrey, J. Kennedy, P. Mombaerts, S. Tonegawa and A. Zlotnik, unpublished). The first control point therefore defines the transition into the TN stage, including CD4 down-regulation, induction of CD25 expression and possible commitment to the T-cell lineage. The latter proposal is supported by the recent report that unlike the CD4^{lo} pre-TN the CD25$^+$TN cells cannot give rise to DC after *in vivo* transfer[26], and there is presently no evidence for a CD25$^+$ B-cell precursor[27].

Control point 2: induction of TCR-β and -γ gene rearrangement

The expression of CD25 (IL-2Rα) is well recognized as a transient step during early T-cell differentiation

Table 1. Characteristics of early thymocyte subsets

Subset	TCR-β locus[18]	TCR-γ locus[18]	Cytokine responsiveness	Cytokine production[a]	Other characteristics
CD4^{lo}CD44$^+$CD25$^-$	Germline	Germline	Unknown	Unknown	Not committed to T lineage[25,26]
CD44$^+$CD25$^+$TN	Germline	Germline	IL-1[a], IL-7[b], IL-7+SCF[18], IL-12+SCF[c], IL-2+IL-7+SCF[e], IL-7+TNFα+TGFβ[c,65,66]	IL-2, IFNγ, TNFα[18]	IL-1 maintains size *in vitro*[c] Large cells (cycling?)[18]
CD44$^-$CD25$^+$TN	Rearranged	Rearranged	IL-1[a], IL-7[b], IL-7+TNFα+TGFβ[c,65,66]	IL-2, IFNγ, TNFα[18]	Smaller cells[18] Some may express TCR-β dimers Require 'β-selection' to progress
CD44$^-$CD25$^-$ CD4^{lo}CD8^{lo}	Rearranged	Rearranged	IL-1+IL-7+IL-2+SCF[40]	None	Become CD3^{lo}CD4$^+$CD8$^+$ *in vitro* (Programmed differentiation)[4]

[a] Cytokines are produced after activation with calcium ionophore and phorbol ester only in the presence of IL-1[18].
[b] IL-7 maintains the viability of CD25$^+$TN cells *in vitro* for up to 7 days, without losing their repopulation potential[29]. CD44$^+$CD25$^+$TN cultured in IL-7 show better viability than CD44$^-$CD25$^+$TN[18]. IL-7 does not induce TCR-β gene rearrangement in CD44$^+$CD25$^+$TN *in vitro*.
[c] D. Godfrey and A. Zlotnik, unpublished observation.
[d] Cells become CD8$^+$ (Refs 65, 66).
[e] G. Kelner and A. Zlotnik, unpublished observation.

(reviewed in Refs 5, 10, 13). However, the role of IL-2 at this stage remains controversial, indeed IL-2 deficient mice show a phenotypically normal thymus[28], although thymic development during fetal ontogeny has not yet been examined. The CD25⁺TN cells were reported mostly to be rearranged (approximately 75%) at the TCR-β locus and producing full-length 1.3 kb TCR-β transcripts[19]. This was interpreted as a second wave of rearrangement in accordance with the observation that approximately half of the previous subset (CD44⁺CD25⁻TN) had already rearranged their TCR-β genes[19]. However, our observation that the subset of CD44⁺CD25⁻TN with rearranged TCR-β genes were c-kit⁻ and unlikely to be early precursors (see above), indicated that TCR-β rearrangement probably begins at the CD25⁺TN stage. Although most studies on these cells have looked at whole CD25⁺TN (for example, Refs 19, 30, 31), they can be further subdivided by CD44 expression[18,22,31] and both the CD44⁺CD25⁺TN and the CD44⁻CD25⁺TN subsets have thymic repopulating ability[18,20]. It has been recently reported that these two populations were functionally distinct[18] and, most importantly, that the CD44⁺CD25⁺TN have not yet rearranged their TCR-β and -γ genes[18]. They do, however, produce both 1.0 kb and 1.6 kb TCR-β transcripts, which probably represent germline transcription. Indeed, germline transcription of TCR-β[32], TCR-γ[33] and TCR-α[34] loci has also been described during early embryonic T-cell development, and may represent a mechanism by which these genes are targeted for rearrangement[35].

The CD44⁺CD25⁺TN express high levels of c-kit and proliferate in response to SCF plus IL-7[18] or to SCF plus IL-12 (D. Godfrey and A. Zlotnik, unpublished). The transition from the CD44⁺CD25⁺TN to the CD44⁻CD25⁺TN stage is accompanied by the rearrangement of both TCR-β and -γ loci, followed by the production of what appear to be full-length 1.3 kb TCR-β transcripts[18]. This concurs with the previous observation that a small proportion of 1.3 kb TCR-β mRNA is first detectable in the whole CD25⁺TN fraction[19]. This transition also leads to c-kit down-regulation, and diminished responsiveness to SCF and IL-7[18], as well as IL-12 (D. Godfrey and A. Zlotnik, unpublished). Suda and Zlotnik[29] reported that IL-7 maintains the viability of CD25⁺TN thymocytes *in vitro*, and that the cells cultured under these conditions retain thymic repopulation potential. As explained above, the IL-7-responsive cells are primarily the CD44⁺CD25⁺TN[18], however, these IL-7-cultured cells retain their TCR-β genes in a germline configuration, even after four days (D.I. Godfrey, J. Kennedy, P. Mombaerts, S. Tonegawa and A. Zlotnik, unpublished). This contrasts a recent report that implicates a role for IL-7 in the induction of TCR-β rearrangement in 14-day fetal thymocytes[36]. However, in this study, IL-7 was the only factor tested that did not shut down the expression of recombination activating genes (RAG)-1 and -2, which were already being transcribed in the starting population. Furthermore, DJ rearrangements of the TCR-β locus are already detected by 14 days of gestation[37]. Since IL-7 is the only known factor that does not disrupt the differentiation potential of TN thymocytes *in vitro*[29], it is possible that IL-7 was the one physiologically appropriate stimulus that maintained the 14-day embryonic thymocytes, while enabling the completion of their rearrangement programming which was already underway. Taken together, we suggest that CD44⁺CD25⁺TN represent the final stage before TCR-β and -γ gene rearrangement. The signal(s) that induce TCR-β and -γ gene rearrangement are therefore delivered at some point between the CD44⁺CD25⁺TN and the CD44⁻CD25⁺TN stages. This process is controlled by as yet unidentified factor(s) present within the thymic microenvironment.

Control point 3: branching of alternative T-cell development pathways

At least two alternative pathways of T-cell development have been described in the thymus (reviewed in Ref. 2), leading either to the production of γδ T cells or TCR-αβ⁺DN T cells. It has been reported[38] that stimulation of early (CD5^{dull}) DN cells with IL-1 and Con-A resulted in the generation of TCR-αβ⁺DN T cells, whereas the same cells stimulated by IL-2 and Con-A generated γδ T cells. Suda and Zlotnik[39] demonstrated that both γδ T cells and TCR-αβ⁺DN cells can develop directly from whole CD25⁺TN after culture in the presence of IL-7.

We recently reported that the CD44⁻CD25⁺TN cells were the last subset with the ability to repopulate the γδ component of lymphocyte-depleted fetal thymic organ cultures (FTOC)[18]. This conflicted with an earlier report by Petrie *et al.*[40] where CD44⁻CD25⁻CD4^{lo}CD8^{lo} cells were shown to repopulate both the αβ (mainstream) and γδ T-cell component of irradiated mouse thymi *in vivo*. However, it is possible that the γδ branch point occurs at the CD44⁻CD25⁺TN stage, and committed αβ and γδ T-cell precursors undergo a phenotypically parallel development step (down-regulation of CD25), before cell surface expression of their respective TCR. This scenario implies that some of the CD44⁻CD25⁻TN cells are αβ precursors, while others are γδ precursors. That the putative CD44⁻CD25⁻TN γδ precursors may be able to expand and repopulate the γδ component of the *in vivo*, but not the *in vitro* thymus, may be due to the different experimental conditions. For example, it is likely that there would be no lymphoid-derived cytokines in the depleted FTOC since CD44⁻CD25⁻TN apparently do not have cytokine producing potential[18].

The precise mechanism(s) regulating γδ *versus* αβ T-cell branching are unknown, but possibly involve transcriptional silencing of TCR-γ genes in αβ T-cell precursors (reviewed in Ref. 41). Many αβ T cells have rearranged TCR-γ genes and conversely, γδ T cells usually have their TCR-β genes in an incompletely rearranged DJ form. According to our model, this places the γδ T-cell branch point at the CD44⁻CD25⁺TN stage, probably before these cells begin to produce full-length (1.3 kb) TCR-β transcripts. It must be pointed out however, that this branch point is based upon the assumption that all TCR-β and -γ rearrangement occurs at the same stage during adult T-cell development, as indicated by Southern blot analyses[18]. Although less likely, we cannot rule out the possibility that a very

minor subset of cells have rearranged both their TCR-β and -γ genes at an earlier stage, and have already branched off toward the γδ T-cell lineage.

It is more difficult to identify a particular stage at which the TCR-αβ⁺DN thymocytes are likely to branch away from mainstream αβ T-cell development. This is possibly due to the fact that the TCR-αβ⁺DN cells are heterogeneous (A. Bean, D. Godfrey, L. Santos, M. Howard and A. Zlotnik, unpublished) and may have multiple origins. In addition to the generation from these cells from CD44⁻CD25⁺TN[39], cells with similar phenotype also develop *in vitro* as a result of TCR-mediated stimulation of immature CD3ᵇᵘCD4⁻/ᵇᵘCD8⁺ thymocytes (immediate precursors to DP)[42], and from CD2 cross-linking of rat DP thymocytes[43]. Therefore, we suggest that the CD44⁻CD25⁺TN stage is a likely branch point for TCR-αβ⁺DN thymocytes, but that later stages may also give rise to cells with this phenotype.

Control point 4: early selection for cells expressing a functional TCR-β chain ('β-selection')

Several recent studies have investigated T-cell differentiation in mice with mutations in either the RAG-1 or RAG-2 genes[44,45], or in individual TCR genes[46–48]. An important conclusion from these studies was that TCR-β gene rearrangement or expression is necessary for αβ thymocytes to differentiate from the TN to the DP stage. Furthermore, the incorporation of a rearranged TCR-β transgene into the RAG-1 or RAG-2 mutant background was sufficient to restore this differentiation step[47,49]. In a collaborative study with Peter Mombaerts and Susumu Tonegawa, we have recently examined the TN thymocytes from RAG-1 or TCR-mutant mice in greater detail and observed that in the absence of TCR-β gene rearrangements, αβ thymocyte development is blocked at the CD44⁻CD25⁺TN stage. Two possible explanations for this blockage can be postulated. The first is that a TCR-β rearrangement event may itself signal the cell to proceed to the next stage, although in this scenario, we would have expected the blockage to occur at the last unrearranged stage (CD44⁺CD25⁺TN). Alternatively, the rearranged TCR-β chain may have to be expressed, possibly on the surface as a TCR-β dimer, since TCR-α is not expressed at the CD25⁺TN stage[19,50]. TCR-β dimers have been described in TCR-β transgenic rearrangement-deficient mice[49,51] and more recently in normal mice during embryogenesis[52]. These have been proposed to consist of two TCR-β chains paired as a homodimer, although more recent evidence points to the existence of a distinct molecule paired with the TCR-β chain (for a detailed review of this area see M. Groettrup and H. von Boehmer, *Immunol. Today* (in press)). These are able to deliver a signal upon ligation[51] and may conceivably mediate the differentiation beyond the CD44⁻CD25⁺TN stage. The expression of a productive TCR-β chain at this stage probably also prevents further TCR-β rearrangements (allelic exclusion) (reviewed in Refs 7, 53) and may also induce TCR-α gene rearrangements and expression. However, TCR-β mutant mice are able to rearrange their TCR-α genes[47], suggesting TCR-β rearrangement and expression is not an absolute requirement for this process. The importance of productive TCR-β expression at an early stage is further supported by a recent study of TCR-α mutant mice[54]. Thymocytes from these mice can differentiate to the DP stage, but no further, due to their inability to be positively selected (in the classical sense) in the absence of a functional CD3/TCR-αβ complex[44,48]. Analysis of TCR-β gene rearrangements in these thymocytes showed a high proportion of productive (in-frame) rearrangements[54], at a far higher level than would be predicted through random recombination. Although it was uncertain as to whether these rearrangements occurred in the majority of thymocytes in these mice rather than a minor subset, this study suggested that a selection event had occurred for cells expressing productively rearranged TCRγβ in the absence of a TCR-α chain.

As described above, CD44⁻CD25⁺TN produce full-length 1.3 kb TCR-β transcripts[19], but do not yet transcribe TCR-α genes[19,50]. Taken together, we propose that TCR-β is expressed at very low levels, probably as TCR-β dimers, on at least a subset of the CD44⁻CD25⁺TN cells. Those cells with functional TCR-β chains are selected for, and allowed to progress to the DP stage. Also consistent with this model is the observation that addition of anti-CD3 to FTOC caused the rapid transition from the CD25⁺TN stage to the DP stage[55], which was proposed to mimic the normal maturation signal delivered through the immature TCR-β/CD3 complex. We will refer to the early selection of TCR-α⁻β⁺ cells as 'β-selection', to distinguish it from the well described phenomenon of 'positive selection' of TCR-α⁺β⁺ cells.

One putative signalling factor in β-selection may be the lymphocyte-specific protein tyrosine kinase p56ˡᶜᵏ. Thymocyte development in p56ˡᶜᵏ-deficient mice is retarded beyond the TN stage, such that there are less than 10% of the wild-type numbers of DP cells, and no mature SP cells[56]. Furthermore, a complete block at the CD44⁻CD25⁺TN stage has been reported in transgenic mice overexpressing a catalytically inactive form of p56ˡᶜᵏ (Ref. 57). Thymocytes from these mice show complete V(D)J rearrangement at the TCR-β locus but the TCR-α locus remains in germline configuration. Conversely, overexpression of the wild-type p56ˡᶜᵏ molecule in developing thymocytes resulted in a specific reduction in TCR Vβ-Dβ rearrangement, but did not inhibit TCR-α rearrangement and transcription, or the progression to the DP stage[58]. The notion that the TCR-β transmits intracellular signals *via* p56ˡᶜᵏ has been further supported through analysis of mice that simultaneously overexpress a catalytically inactive p56ˡᶜᵏ and a TCR-β transgene[59]. In these double transgenic mice, but not in single TCR-β transgenic mice, there was no allelic exclusion at the endogenous TCR-β locus. Taken together, these observations strongly suggest that p56ˡᶜᵏ regulates development through control point 4, independently of CD4 and CD8 expression[60]. It remains to be shown whether functionally rearranged TCR-β chains need to be expressed at the cell surface to mediate β-selection. If surface expression is required, it is possible that interaction with an as yet undefined intrathymic ligand may take place.

By definition, control point 4 requires complete V(D)J rearrangement of the TCR-β locus. It follows that this probably occurs after the branching of γδ T cells (control point 3), which, as discussed above, do not usually reach this level of TCR-β rearrangement.

Control point 5: induction of CD4 and CD8 expression, and TCR-α rearrangement and expression

The transition from the $CD44^-CD25^+$TN to the $CD44^-CD25^-$ stage involves the induction of CD4 and CD8 expression. The latter subset is often referred to as the last TN stage, but as already mentioned, most of these cells are transcribing and expressing low levels of CD4 and CD8 ($CD4^{lo}CD8^{lo}$)[61]. This transition is also accompanied by the first round of TCR-α gene rearrangement and expression[19,50], which eventually leads to the expression of CD3/TCR-αβ at the DP stage. This step is also under the control of the thymic microenvironment, since isolated $CD25^+$TN are unable to differentiate to the DP state *in vitro*[29,62]. As discussed above, the progression from $CD44^-CD25^+$TN to the $CD44^-CD25^-CD4^{lo}CD8^{lo}$ stage may be regulated, at least in part, by signal(s) mediated *via* the expression of a productive TCR-β chain by the $CD44^-CD25^+$TN cells. However, since TCR-α rearrangement can occur in the absence of TCR-β expression[47], and CD4 and CD8 can be induced on TCR-$β^-$ SCID mouse thymocytes[63,64], other factor(s) are also likely to be involved, including cytokines and other cell surface molecules. For example, transforming growth factor (TGF)β and tumour necrosis factor (TNF)α can induce CD8α-chain expression (but not CD4) in $CD25^+$TN thymocytes cultured with IL-7[65,66]. We therefore propose that this transition represents a separate control point, which may or may not be closely linked to the previous control point 4.

The last step to be covered in our early T-cell development model is the generation of the DP thymocytes from the $CD44^-CD25^-CD4^{lo}CD8^{lo}$ cells. This can proceed either directly, or *via* immature CD4 or CD8 single positive subsets (reviewed in Ref. 67). These subsets have not been included in Fig. 1 for simplicity sake. When $CD44^-CD25^-CD4^{lo}CD8^{lo}$ cells are isolated and cultured, they spontaneously differentiate into DP cells within 24 h, in a process termed programmed differentiation[61,68,69]. These cells have probably received all the necessary signals at control point 5, and simply represent a transient step between the TN and DP stages of intrathymic T-cell differentiation.

Conclusion

This review presents an updated model for early T-cell differentiation, incorporating five separate control points of pivotal importance to the fate of developing thymocytes (Fig. 1). The first control point in T-cell development involves commitment to the T-cell lineage. The subsequent rearrangement and expression of individual TCR genes is regulated by the thymic microenvironment. These events probably determine sublineage diversification (αβ *versus* γδ), and further differentiation. Several studies now strongly suggest that there is an early selection point (β-selection) during TN development that serves as a 'quality control' step for productive TCR-β rearrangements. The exact nature of the signals that regulate these events is unknown, but we have now defined the stages at which they are probably acting. The identification of these control points should provide a strong foundation for the determination of the mechanisms that regulate early T-cell development.

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